RESEARCH ARTICLE

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Isolation of pigment producing organisms from unpolished *Oryza sativa* from India

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ABSTRACT

Synthetic pigments generally have high toxicity content; it can be allergic, carcinogenic and even lead to gastrointestinal problems when consumed via food. These demerits of synthetic pigments led to research towards natural pigments or colors from natural source like microorganisms which is considered one of the best alternative natural sources for bio pigments. Isolations of pigmented colonies were carried out from unpolished raw rice, followed by a viable count in a phosphate buffer of pH 7.2 by pour plate and spread plate method. Organisms producing three different pigments – yellow, orange and pink were isolated. Two types of these pigments were intracellular while one was diffusible. Intracellular pigments – yellow and orange color pigments were extracted from producing cells by acetone/methanol solvent method. Extraction of diffusible pigment was carried out by using ethanol and petroleum ether method. And then antimicrobial activities of the isolated pigments were carried out by the agar cup method against *S. aureus, E. coli* as test organisms. The isolated pigments can be used in lip balm, nail paints, eco-friendly paints, etc.

Keywords: Natural pigments, methods of extraction, antimicrobial activity

1. INTRODUCTION

Colors are the major aspect of things that results from different quality of light being reflected or transmitted by them in a visible region of the electromagnetic spectrum. Color(s) can be broadly categories into two types – Synthetic color(s) and natural color(s). Synthetic color(s) are chemical substances which impart its own color and use for coloring different things based on their property of reflecting and absorbing capacity, and prepare in laboratories [1]. These Synthetic color(s) have advantages as well as disadvantages; some of the most prominent merits include water solubility. These colourants easily get dissolve in water whereas it is not seen in natural pigments. Synthetic colors have more glow and more color gradient which increase the chance of more choices, and helps in attraction. Synthetic colors have high and quick dyeing capacity or fastness in less quantity, and availability is also high therefore cheaper in cost [2].

But Synthetic color(s) also have adverse effects on environment which become a matter of concern in today's world where environment protection is the first priority of all [3]. It effects the marine life when dispose in water bodies without any proper or efficient sewage treatment, effects soil microflora, degrade health of workers, and even causes gastrointestinal problems when consume [4]. So, these demerits of synthetic color(s) led to research towards natural color(s) [5].

Natural pigments are organic and can be extracted from natural sources like vegetables, animals, insects, and microorganisms [6-7]. Extraction of natural pigment from microorganisms is considered as one of the best alternative natural sources for bio pigment [8-9]. Few microorganisms have beneficial as well as adverse effects on human health.

Rice is a staple diet of most Asian countries and consumed in large quantities [10]. It is scientifically proven that eating unpolished rice is consider as healthy because it contains high fibers, high in micronutrients like high in selenium, phosphorus etc, these micronutrients are needed by our body on the regular basis, consumption of whole grain foods now a days being promoted because the regular consumption of whole grain foods (such as rice) reduces risk of chronic diseases [11]. Unpolished rice associated with many wide ranges of nutrigenomic implications such as anti- diabetics, anti- cholesterol, anti – oxidant and also has cardio-protective property [12]. White rice contains mainly the starchy endosperm, the removal of rice bran leads to a loss of nutrients during milling about 85% of fat, 15% of protein, 90% of calcium, 70% of phosphorus, 70% of vitamins like (B1, B2, B3,) are removed [13]. Still people prefer to consume white rice because its cooking is easier as compare to unpolished rice due to slow water absorption and palatability quality of unpolished rice is inferior to white rice [14]. And at same time these unpolished rice is prone to high amount of microbes which can have beneficial as well as adverse effects on human health, like food poisoning and other gastrointestinal tract problems [15]. Therefore examining the microbial load and looking for harmful microbes become a matter of concern.

This study focuses on isolating the pigment(s) from unpolished rice and checks its bioactive efficacy.

2. METHOD AND MATERIAL

2.1. Sample collection and preparation

Sample of unpolished rice (*Oryza sativa*) was collected from Local super market of Mumbai, India. 12(g) of rice sample was measured and grinded using phosphate buffer (pH 7.2). The stock solution and its dilkution (upto 10^{-10}) was stored for further use.

2.2. Incubation of organism

The 1 ml of prepared sample dilution was evenly spread with nutient broth by pour plate and 0.1 ml on solidified medium for spread plate methods. The plates were allowed to solidify and then incubated it for 48 hours at 37°C.

2.3. Isolation of pigment producing organisms

After incubation different colour or pigmented organisms were observed on plated. Most common were yellow, orange and pink. Three pigments were further purified and isolated in nutrient broth.

2.4. Characterisation of isolated organisms

The isolated were tested with Gram staining method and and morphological characteristics were done.

2.5. Extraction by acetone:methanol solvent method

The organisms in nutrient broth were centrifuged at 1000 RPM for 30 min to disociates the cell and release the pigments. The cell pellets are inoculated with acetone: methanol solvent in (4:3(v/v) ratio). Again centrifuged the tubes at 1000 rpm for 10 min to make the cells pigmentless, the solution was poured in sterile petri plate and left overnight for evaporation, After evaporation, the crystals of pigments were dissolved in DMSO solvent and kept for further use.

2.6. Extraction of diffusible pigment by ethanol and petroleum ether method

The organisms were grown in nutrient broth under shaking condition for 48 hours at 25°C, then 20ml of ethanol was added. The solution was kept under shaking condition for 3 hours at 25°C, then added 5ml of petroleum ether. The tube was kept undisturbed for 1 hour to get the layers separated followed by removal of the bottom layer in sterile test tube and use it for further tests.

2.7. Antimicrobial detection activity test

The assay was carried out to evaluvate the presence of any bioactive compound in the extracted pigments agianst *S. aureus* and *E.coli* as test organisms. The test organisms *S. aureus* and *E. coli* (0.3ml each) were poured with molten Muller Hinton agar in sterile petri plates. Wellwere prepered using cork boarer on each plate. the extracted pigments were added into the wells. DMSO and petroleum ether was used as control for the test. The plates were incubated at 37 °C for 24-48 hours.

3. RESULT AND DISCUSSION

3.1. Isolation of pigment producing organism

The isolation of orgainsm was done successfully. The isolation was done by pouar plate nad spread plate method. In pour plate method dilutions (10^{-4},10^{-5},10^{-6}) plates and from surface spread method dilutions (10^{-3}, 10^{-5}, 10^{-7}) plates showed the pigment producing organisms. Among the microbial colonies on nutrient agar, three distinctive colonies were found to produce pigments. There were three different types of pigment producing organisms (yellow, pink and orange) and shape were observed. Microbial pigments are the characteristic feature of some bacteria to produce pigments which may be useful in identification. Bacterial pigments offer promising avenues for various applications due

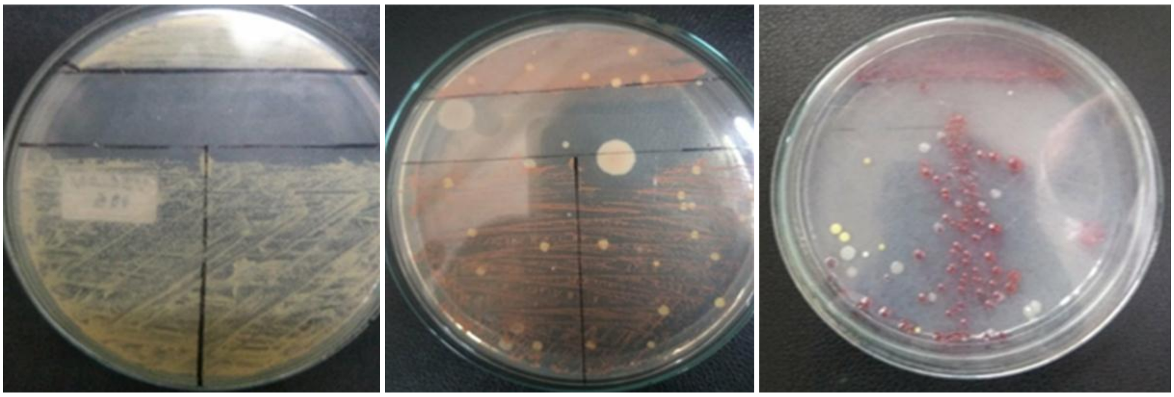

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Image 1. Isolated pigment-producing organisms

to their better biodegradability and higher compatibility with the environment [17].

3.2. Characterisations of pigment producing organisms

It was found that all the three yellow, orange and pink colour pigment producing organisms were Gram positive in nature and Diplococci, Rods and cocci in singles in arrangement respectively (figure 2). All the cells were flat, circular, opaque and had entire margin. The result was similar with other studies conducted on other food item [16-17]. Bhat, S. V *et al*., (2013) revealed that yellow and orange coloured pigment producing organism were isolated from food item like bread, samosa etc. The yellow coloured pigment organism belonged to *Micrococcus* family and the orange

Table 1. Morphological Characteristics of the three pigment colonies

Color/ Characteristics	Yellow pigment	Orange pigment	Pink pigment
Color	Yellow	Orange	Pink
Size	2-3 mm	0.5 – 1 mm	2 – 4 mm
Shape	Circular	Circular	Circular
Elevation	Flat	Flat	Flat
Opacity	Opaque	Opaque	Opaque
Margin	Entire	Entire	Entire
Gram nature	Gram positive, diplococcic	Gram Positive, Rods	Gram positive, cocci in singles

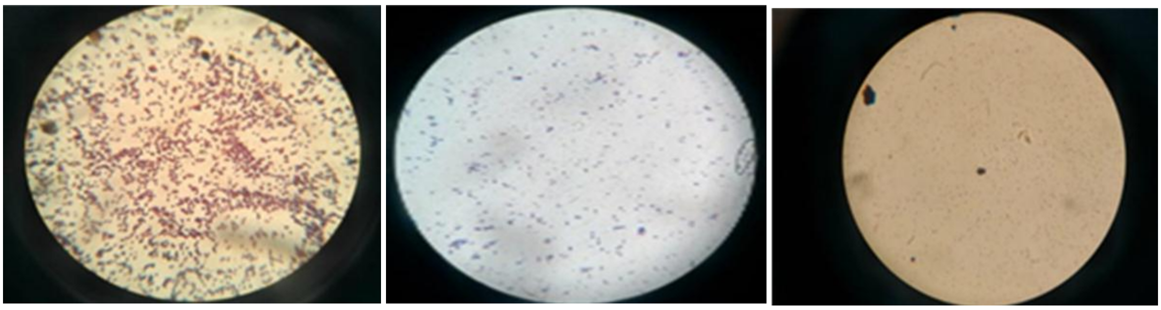

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Figure 2. Pigment producing organisms under microscope

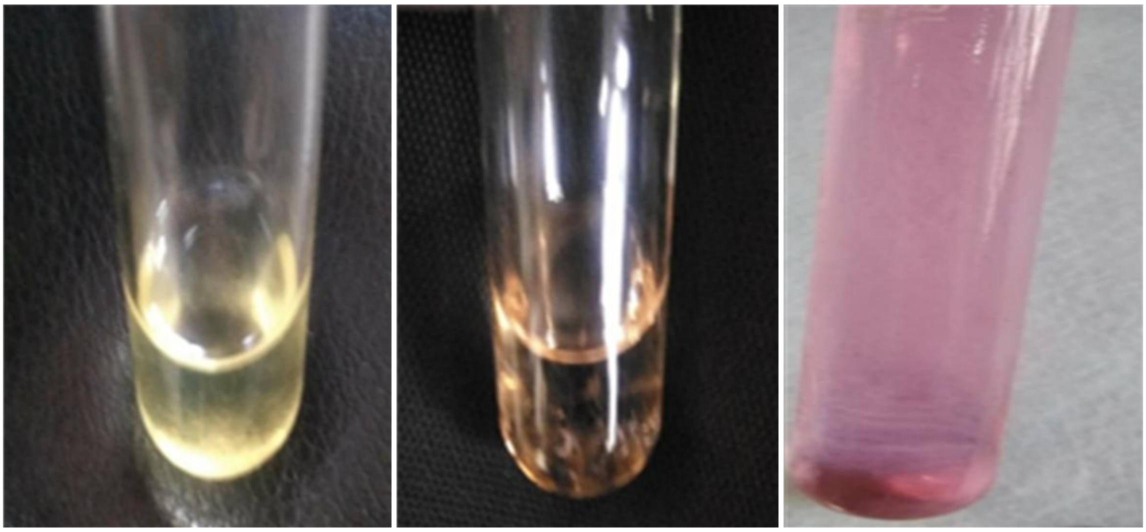

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Figure 3. The extracted pigment from pigment producing organisms

coloured organism was *M. nishinomiyaensis* [18]. The results of this study were similar to our study.

3.3. Extraction of pigments

The yellow and orange pigments were intracellular and extracted by acetone/methanol solvent method, whereas the pink pigment was diffusible in nature and extracted by treating it with ethanol and petroleum ether method. The extraction method used for extracting yellow and orange pigment was not found to be an efficient method because of less feasibility [21].

3.4. Antimicrobial assay

In antimicrobial detection test, no zone of inhibitions were observed against the test organisms *S. aureus* and *E. coli* , which indicates no bioactive substances present in extracted pigments this implies that pigments can't be used in pharmaceutical purposes but can be used in lip balm, nail paints and eco- friendly products (in which antimicrobial property is not needed). Similar results were obtained

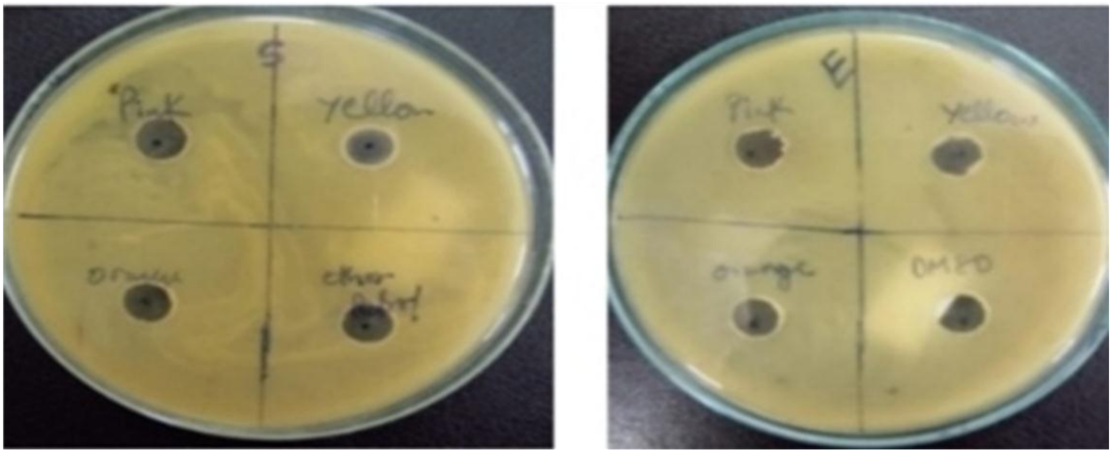

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Figure 3. The antimicrobial test results against the test organisms *S. aurus* and *E. coli*

from study conducted by Colien, F. E. (1935) where he performed antigenic test. No relationship was found between this yellow pigment-producing coccus and *Micrococcus aureus*. No cross agglutination occurred between the variants [16].

Most studies suggest that some bacteria have efficacy and potential in clinical applications. The pigments from bacteria and fungus has been used in treating several diseases and the also have certain properties like anti-biotic, anticancer, and immunosuppressive compounds [22].

4. CONCLUSION

From this study it was observed from this study that isolating pigment producing bacterium may or may not have a bioactive substances and different extraction methods should be used for different pigments based on their nature and property. But extraction methods need to focus on feasibility, because extraction methods used in this study are not having high feasibility on large scale production.

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6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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